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Determination of Phthalates Migrating from Plastic Containers into Beverages

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Abstract The determination of phthalates in beverages (soda, lemonade, cola, mineral water) sold in Turkish markets was carried out using gas chromatography-mass spectrometry (GC-MS). The mean phthalate concentrations were determined to be between 0.095 and 0.633 mg/L in soda, 0.018 and 1.219 mg/L in lemonade, 0.019 and 1.123 mg/L in cola, and 0.085 and 0.312 mg/L in mineral water. bis(2-Ethylhexyl) phthalate (DEHP) showed the highest level of migration into beverages. Furthermore, the influence of the type of preservative (sodium benzoate, potassium sorbate, sodium benzoate+ potassium sorbate) and storage time were determined.

Keywords Phthalates · Beverages · GC-MS · Endocrine disruptor

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Introduction

Phthalates are well-known compounds in the form of dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid, the so-called phthalate esters (PAEs). The unique properties of higher molecular weight PAEs, which primarily include stability, fluidity, and low volatility, make them highly suitable as plasticizers (Chen 2004). This is why they can be found in a broad array of commercial products, including plastics, cosmetics, nail polishes, hair sprays, perfumes, fishing lures, children's toys, medical devices, food packaging, medications, building materials, home furnishings, transportation, clothing, caulk, dope, paints, adhesives, and lubricants made with polyvinyl chloride plastics.

Phthalates are considered as an endocrine disrupter because they display a variety of toxic effects in animal studies including decreased fertility in females (Biscardi et al. 2003), fetal defect and reduced survival of offspring (Gray et al. 2000), altered hormone levels (Thompson et al. 2004), uterine damage (Seidlova-Wuttke et al. 2004), and male reproduction abnormalities such as reduced sperm production and mobility (Sharpe et al. 1995), Sertoli cell damage (Heindel and Powell 1992), and cryptorchidism and hypospadias (Skakkebaek et al. 2001). The effects of human exposure to phthalates have not been fully studied (Montuori et al. 2008).

Regulations governing the use of plasticizers in food contact applications vary from country to country. In the UK, the acceptable concentrations have been set for bis(2-ethylhexyl) phthalate (DEHP) at 0.05 mg/kg body weight/day, butylbenzyl phthalate (BBP) at 0.1 mg/kg body weight/day, dibutyl phthalate (DBP) at 0.05 mg/kg body weight/day, and diethyl phthalate (DEP) at 0.2 mg/kg body weight/day (MAFF 1996). In Europe, the total tolerable daily intake of total phthalates per person has been estimated to be 0.3 mg/kg

body weight (Balafas et al. 1999). In the Turkish Food Codex, the acceptable limits of the phthalates in food are summarized as below:

- DBP, do not exceed 0.3 mg/kg food
- BBP, do not exceed 30 mg/kg food
- DEHP, do not exceed 1.5 mg/kg food
- Diisononyl phthalate (DINP), do not exceed 9 mg/kg food.

Phthalates are not bound chemically in the plastics and can consequently penetrate these materials and migrate into food that comes into contact. The presence of phthalates in packaging materials and their migration into packaged foods have been confirmed by a number of authors (Balafas et al. 1999; Bosnir et al. 2007; Cavaliere et al. 2008; Ceretti et al. 2010; Holadová et al. 2007; Nanni et al. 2011; Ostrovský et al. 2011; Sannino 2009). The aim of the study is to determine the phthalate levels in beverages (soda, lemonade, cola, mineral water) sold in Turkish markets.

Materials and Methods

Reagents and Standards

Dimethyl phthalate (DMP), DEP, DBP, dipropyl phthalate (DPP), BBP, DEHP, dioctyl phthalate (DOP), and DINP were obtained from Fluka (Buchs, Switzerland). All the phthalate esters were more than 99 % pure.

A stock standard solution of 1,000 mg/L of each compound was prepared in acetonitrile. Working standard solutions of 100 mg/L were prepared weekly in acetonitrile. Stock and working standards were stored at 4 °C in the refrigerator.

Calibration standards were prepared in the range of 0.01–2 and 2–10 mg/L. In all cases, the correlation coefficients of linear functions were >0.995. The calibration curves were created from six calibration standards.

Collection of Samples

Ten different brands from each item beverages (soda, lemonade, cola, mineral water, and orange-flavored soda) were taken from different local markets in Turkey.

Sample Preparation

After the beverage had been degassed in an ultrasonic bath for 5 min, an aliquot of either 20 mL was extracted twice with 10 mL of dichloromethane in a separating funnel. A sodium sulfate column was prepared by packing sodium sulfate (1 g) in turn into a glass column fitted with fritted glass disks at the bottom (15 mm×110 mm). The column was washed with dichloromethane (5 mL) before use. The extract was passed through the column and evaporated to a small volume of approximately 1–2 mL at 40 °C under vacuum. Then, it was transferred to a vial and was analyzed by gas chromatography-mass spectrometry (GC-MS) (Keith et al. 2000).

GC-MS Analysis

GC-MS analysis was performed on a Hewlett-Packard 6890 instrument. A Hewlett-Packard HP-5MS fused silica capillary column (cross-linked 5 % methyl silicone of 30 m×0.25 mm I.D. and phase thickness of 0.25 μm) was used. The GC temperature program was as follows: initial temperature of 50 °C, hold for 5 min; increased to 90 °C at 2 °C/min, hold for 3 min; and then to 200 °C at 10 °C/min, hold for 10 min. The total run time was 49 min. Helium was used as carrier gas at a flow rate of 1.2 mL/min. The samples were injected in the splitless mode. The sample volume in the direct injection mode was 1 μL. The MS conditions were the following:

- Instrumentation, HP 6890/5972 mass spectrometer
- Transfer line temperature, 280 °C
- Source temperature, 250 °C
- Electron energy, 70 eV
- Constant flow, 1 mL/min
- Pressure, 22.39 psi

Quality Control and Quality Assurance

The amounts of phthalates were determined by comparing their peak areas with those of standards in GC-MS. All analyses were repeated three times for each sample. A blank version of the test performed on the beverages was conducted every day using ultrapure water. All of the blank values were averaged, and the average value was subtracted from the detected phthalate values. The limit of detection (LOD) was determined to be three times the standard deviation of the blank test values. The limit of quantification (LOQ) was taken as three times the LOD. The values of retention time, correlation coefficient, LOD, and LOQ of examined phthalates are listed in Table 1.

The laboratory blank is very important since the contamination is a major problem in the analysis of phthalates, especially from unclean plastic-containing glassware, organic solvents, and many items in laboratory settings (Mohamed and Ammar 2008). In this study, special care was taken to avoid the contact of reagents and solutions with plastic materials. Laboratory glassware was washed prior to use with ultrapure water and dried at 250 °C.

Table 1 The values of retention time, correlation coefficients, LOD, and LOQ of examined phthalates

Compound name	Retention time (min)	Correlation coefficient (R^2)	LOD (mg/L)	LOQ (mg/L)
DMP	34.51	0.998	0.002	0.006
DEP	36.63	0.999	0.002	0.006
DPP	38.98	0.999	0.003	0.009
BBP	40.76	0.996	0.002	0.006
DBP	41.24	0.998	0.004	0.012
DEHP	43.47	0.996	0.007	0.021
DINP	43.53	0.997	0.009	0.027
DOP	43.95	0.998	0.002	0.006

Results

Under the applied GC-MS conditions, the retention times of DMP, DEP, DBP, DPP, BBP, DEHP, DOP, and DINP were 34.51, 36.63, 41.24, 38.98, 40.76, 43.47, 43.95, and 43.53 min, respectively. The chromatogram of phthalate standards was given in Fig. 1.

The phthalate levels of the examined samples were presented in Table 2. The mean phthalate concentrations were determined to be between 0.095 and 0.633 mg/L in soda, 0.018 and 1.219 mg/L in lemonade, 0.019 and 1.123 mg/L in cola, 0.085 and 0.312 mg/L in mineral water, and 0.018 and 0.218 mg/L in soda (orange flavored). DEHP showed the highest level of migration into beverages. A very low level of DOP was detected in two group drinks. The chromatogram of mineral water was given in Fig. 2.

The influence of the type of preservative (sodium benzoate, potassium sorbate, sodium benzoate+potassium sorbate) used in the product manufacture was investigated. The highest phthalate levels were measured in soda samples with sodium benzoate and potassium sorbate used as preservative. The phthalate concentrations in the soda samples preserved with potassium sorbate were found to be similar to the phthalate concentrations in the soda samples preserved with sodium benzoate (Table 3).

The influence of storage time to the phthalate migration was investigated. Related table are shown in Table 4. These data indicate that the phthalate concentrations were increased depending on storage time.

Discussion

US Environmental Protection Agency (EPA)'s maximum contaminant level for DEHP in the bottled water is 0.006 mg/L (EPA 2009). The EU and the World Health Organization (WHO) accepted a permissible limit for drinking waters as 0.008 mg/L of DEHP (European Union Council 2001; WHO 2008). On the other hand, the Japanese authority redefined the permissible DEHP limit in drinking water in 2001 as 0.100 mg/L (Hirose et al. 2004). Bottled water was not examined in our study; however, the concentration of DEHP in soft drinks was 0.248–1.123 mg/L. These concentrations are lower than the permissible limits. BBP concentration was 0.018 to

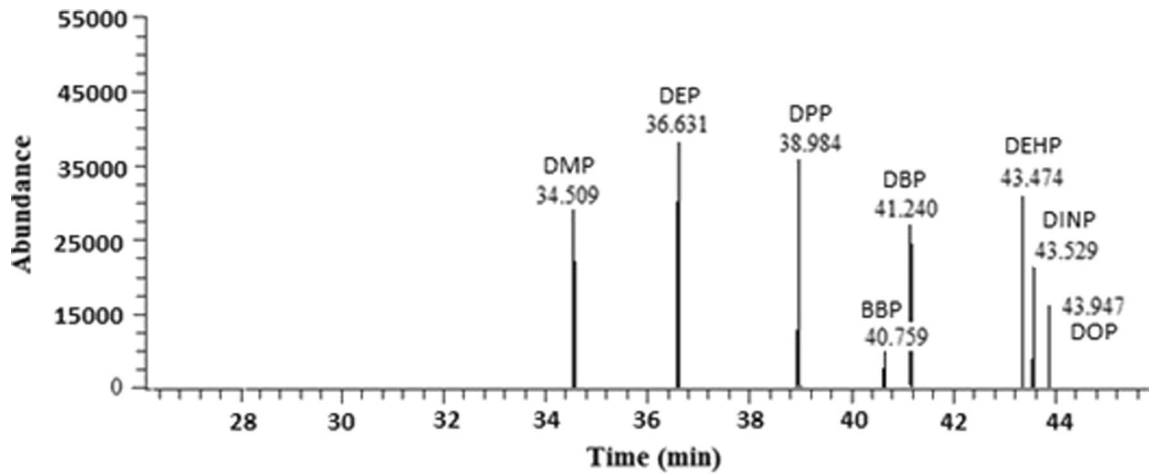

Fig. 1 The chromatogram of phthalate standards

Table 2 Phthalate migration, mean individual, and pool levels in beverages kept in plastic containers ($N=3$)

Samples		Phthalate migration, mean individual, and pool levels (mg/L)[a]								
		DMP	DEP	DBP	DPP	BBP	DEHP	DOP	DINP	Total
Soda	Min	0.086±0.01	0.092±0.01	0.123±0.01	0.184±0.01	0.116±0.01	0.633±0.02	nd	nd	0.486±0.02
	Max	0.118±0.01	0.133±0.01	0.238±0.01	0.303±0.01	0.116±0.01	0.633±0.02	nd	nd	1.516±0.04
	Mean	0.095±0.01	0.111±0.01	0.162±0.01	0.222±0.01	0.116±0.01	0.633±0.02	nd	nd	0.739±0.02
Lemonade	Min	0.029±0.01	0.016±0.01	0.082±0.01	0.038±0.01	0.018±0.01	0.073±0.01	nd	0.580±0.01	0.358±0.01
	Max	0.151±0.01	0.153±0.01	0.198±0.01	0.212±0.01	0.018±0.01	2.060±0.04	nd	1.858±0.04	4.302±0.06
	Mean	0.092±0.01	0.082±0.01	0.162±0.01	0.135±0.01	0.018±0.01	1.045±0.03	nd	1.219±0.03	1.206±0.03
Cola	Min	0.036±0.01	0.032±0.01	0.044±0.01	0.060±0.01	0.359±0.01	0.387±0.01	0.019±0.01	0.122±0.01	0.558±0.01
	Max	0.237±0.01	0.270±0.01	1.500±0.03	0.533±0.01	0.801±0.01	2.312±0.04	0.019±0.01	0.836±0.01	3.442±0.03
	Mean	0.105±0.01	0.123±0.01	0.367±0.01	0.242±0.01	0.580±0.01	1.123±0.03	0.019±0.01	0.479±0.01	1.697±0.04
Mineral water	Min	0.077±0.01	0.071±0.01	0.090±0.01	0.126±0.01	0.917±0.01	0.239±0.01	nd	0.093±0.01	0.426±0.01
	Max	0.106±0.01	0.092±0.01	1.737±0.01	0.455±0.01	0.917±0.01	0.261±0.01	nd	0.093±0.01	2.033±0.02
	Mean	0.087±0.01	0.085±0.01	0.312±0.04	0.218±0.01	0.917±0.01	0.248±0.01	nd	0.093±0.01	0.754±0.01
Orange-flavored soda	Min	0.066±0.01	0.048±0.01	0.073±0.01	0.105±0.01	nd	nd	0.018±0.01	0.200±0.01	0.282±0.01
	Max	0.140±0.01	0.120±0.01	0.116±0.01	0.280±0.01	nd	nd	0.018±0.01	0.237±0.01	0.630±0.01
	Mean	0.074±0.01	0.079±0.01	0.091±0.01	0.156±0.01	nd	nd	0.018±0.01	0.218±0.01	0.442±0.01

nd not detected

[a] Average of triplicates±standard deviation

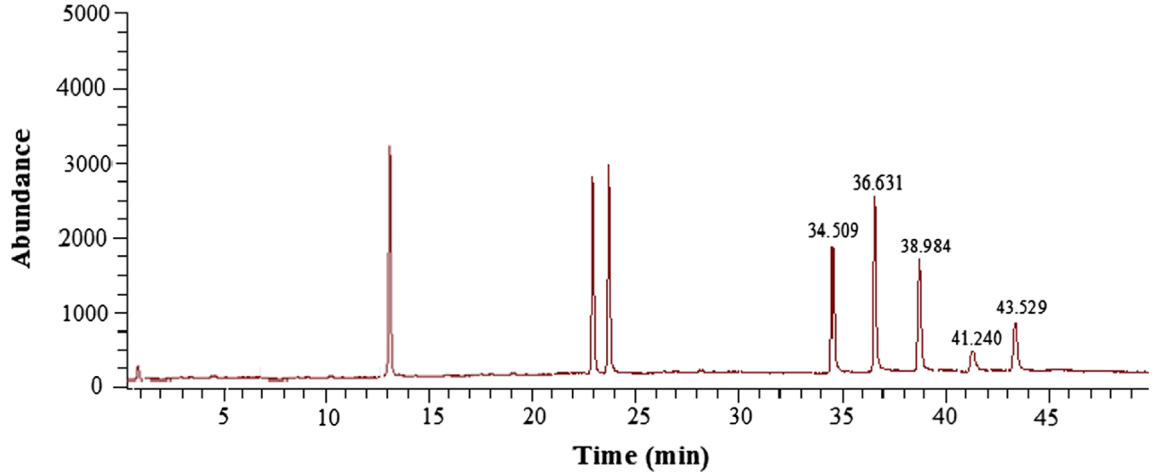

Fig. 2 The chromatogram of mineral water

0.917 mg/L (Table 2). In a previous study, the highest value of BBP was found in bottled water stored at 4 °C (4.592± 3.081 μg/L) (Al-Saleh et al. 2010). US EPA has suggested a maximum contaminant level of 0.100 mg/L for BBP in drinking water (US EPA, http://www.masterwater.com/main/epa_regs.asp). BBP concentrations in lemonade, cola, soda, and orange-flavored soda are still much higher than these high limits. Al-Saleh et al. (2010) found that the maximum DEP value in bottled waters is 0.610–1.778 μg/L. Our value (0.092–0.270 mg/L) was higher than the maximum values reported by Al-Saleh et al. (2010). The DEP levels in the current study were also higher than the DEP mean of 0.17 μg/L reported by Montuori et al. (2008). The study did not include tap water and bottled water, but soft drink was tested. The level of phthalate of soft drink which contains a large number of additives should be different from that of water. The total phthalate concentration also increased as the duration of contamination in soft drinks was longer (from 0.880 to 2.898 mg/L; from 1 to 11 months, for cola drink).

DEHP level in water samples contained in polyethylene terephthalate (PET) bottles increased from a level of 400 ng/L during storage of 1 to 8 months to a high level of 3,200 ng/L in the same water sample after storage of 9 to 12 months (Biscardi et al. 2003). Another researchers did not find a difference in the concentrations of phthalates (DEP, DBP, and DEHP) in water from PET bottles exposed to different storage conditions (15 or 30 days, 15–40 °C) (Amiridou and Voutsa 2011). In our study, the level of phthalate increased as the duration of exposure in all beverages (0.880 mg/L in the 1st month and 2.898 mg/L in the 11th month for cola). Exposure time is short in the study of Amiridou and Voutsa (2011), while the duration of phthalate exposure is long in the study of Biscardi et al. (2003) and our studies. The main reason of phthalate migration may be exposure time rather than other factors such as temperature, humidity, light, pH, and additives.

The nearest phthalate levels to our study in the literature were displayed by Bosnir et al. (2007). They compared the

Table 3 The total levels of phthalate migration to soda samples according to the type of preservative (N=3)

Soda samples	Preservative	Total phthalate concentration (mg/L)[a]
S1	Sodium benzoate	0.558±0.01
S2	Sodium benzoate	0.486±0.01
S3	Sodium benzoate	0.563±0.01
S4	Potassium sorbate	0.573±0.01
S5	Potassium sorbate	0.495±0.01
S6	Potassium sorbate	0.570±0.01
S7	Sodium benzoate+potassium sorbate	1.516±0.03
S8	Sodium benzoate+potassium sorbate	1.508±0.03
S9	Sodium benzoate+potassium sorbate	1.498±0.03

[a] Average of triplicates±standard deviation

Table 4 Phthalate concentrations and expiration date of investigated foods (N=3)

Foods	Expiration date	Exposure time (months)	Phthalate concentration (mg/L)[a]
Lemonade	March 2012	16	1.666±0.02
Lemonade	October 2013	1	0.395±0.01
Cola	May 2012	11	2.898±0.03
Cola	May 2013	1	0.880±0.01
Mineral water	April 2012	16	2.033±0.03
Mineral water	June 2013	1	0.426±0.01

[a] Average of triplicates±standard deviation

concentrations of total and various phthalates in PET-bottled soft drinks preserved with sodium benzoate and/or potassium sorbate. They reported that total phthalate concentration detected in the soft drinks was 0.116–0.819 mg/L. In our study, the levels of total phthalates were 0.739, 0.442, and 1.697 mg/L (soda, cola, and orange-flavored soda, respectively). There was no difference between preservatives such as sodium benzoate and potassium sorbate; however, soft drinks with the use of both preservatives have higher phthalate migration. Researchers found that the highest phthalate levels were measured in soft drinks with potassium sorbate used as preservative (0.819 mg/L), followed by 1.5 times lower levels in drinks preserved with a combination of sodium benzoate and potassium sorbate (0.542 mg/L) and 7 times lower levels in drinks preserved with sodium benzoate (0.117 mg/L). They reported that the phthalate level found in mineral water samples free from preservatives was as low as 0.020 mg/L (Bosnir et al. 2007). Very high levels of phthalates were found in mineral water. The reason for this can be within the preservatives (potassium sorbate). The Food and Drug Administration lists sodium benzoate and potassium sorbate as substances that are generally recognized as safe with a maximum permitted concentration of 0.1 % in accordance with good manufacturing practices (FDA, http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/ucm191033.htm). Similarly, sodium benzoate and potassium sorbate levels in foods are regulated in Europe by the European Commission Health and Consumer Protection Directorate with a limit of 150 mg/L for sodium benzoate and 300 mg/L for potassium sorbate in nonalcoholic drinks (EC, http://ec.europa.eu/food/fs/sfp/addit_flavor/flav11_en.pdf). Can et al. (2011) found the amount of sodium benzoate to be 4–170 mg/L and the amount of potassium sorbate to be 7–144 mg/L in soft drinks sold markets in Turkey. Values do not exceed the legal limits, but the values are very close to the limit. The reason of the conflict between our study and that of Bosnir et al. (2007) may be the difference in the amount of preservatives in soft drinks.

Phthalate-contaminated food products in Taiwan in 2011 were a major event (Wu et al. 2012). The excessive amount of phthalates was added to foods as a substitute emulsifier. Sports drinks, fruit beverages, tea drinks, fruit jam or jelly, and health food or supplements in tablet or powder form were contaminated with di-(2-ethylhexyl) phthalate and/or diisononyl phthalate. A total of 4,076 retail violations were found in inspections of 49,652 store in Taiwan (Kang et al. 2012). Approximately 900 different food products were affected from contamination. According to data from a small sample, DEHP and DINP concentrations ranged from 9.1 to 34.1 ppm and from 5.2 to 7.9 ppm in tainted sports drinks, and DEHP ranged from 2.4 to 14.6 ppm in fruit beverages, respectively. Taiwan government enacted a rule of phthalate-free certification for all food products in the country. The legal concentrations of DEHP, DIDP, DNOP, DINP, DBP, and BBP were considered below 1 ppm. Our total phthalate levels were 0.442–1.697 mg/L in soft drinks. The phthalate amounts of beverages sold in Turkish markets was much lower than those sold in Taiwan.

In a person weighing 70 kg and drinking daily 1,000 mL of lemonade with 0.018 mg/L of BBP, 0.198 mg/L of DBP, 2.060 mg/L of DEHP, 0.153 mg/L of DEP, and 4.302 mg/L of total phthalates corresponding to the maximum concentration values of these phthalates investigated in the lemonade samples in this study, its estimated intake of BBP (7 mg/day), DBP (3.5 mg/day), DEHP (3.5 mg/day), DEP (14 mg/day) would not exceed the MAFF values currently set for phthalates.

As a conclusion, we can say that phthalate is not thought to create a risk factor for human health in Turkey at the present time.

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